Neuromuscular Transmission: Inhibition by Manganese Ions

Abstract. *Manganese ions are potent blocking agents of synaptic transmission at the neuromuscular junction in the frog. The main site of action is the presynaptic nerve terminal, where the ions decrease the amount of transmitter liberated by a nerve impulse. The inhibition produced by manganese is reversible.*

Calcium ions play a key role in a large number of biological processes (*1*); in some of these manganese ions serve as inhibitors (*2*). After depolarization of nerve fibers, there is an influx of calcium ions into the fibers. This influx of calcium ions can be separated, pharmacologically, into two distinct components: an early phase that can be suppressed by tetrodotoxin, and a late phase that can be blocked by manganese ions (*3, 4*). Since this late calcium flux is presumably associated with liberation of transmitter from nerve endings (*4*), we examined the effect of manganese ions on neuromuscular transmission. We report here that manganese ions are a potent inhibitor of neuromuscular transmission and that their main effect is on the presynaptic terminals of motor nerves where they inhibit quantal release of acetylcholine.

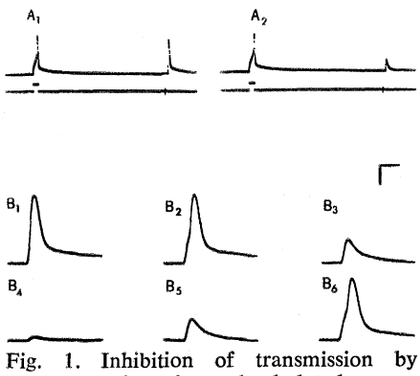

Fig. 1. Inhibition of transmission by manganese ions in a detubulated neuromuscular preparation. Tracing A_1 shows control action potentials. Upper trace, intracellular voltage recording; lower trace measures the current passing through a second microelectrode. The initial response in trace A_1 is a "direct" action potential elicited by a depolarizing pulse, and the second response is an "indirect" action potential after nerve stimulation. Tracing A_2 was taken in the same way as was A_1, but 5 minutes after the addition of 1 m*M* $MnCl_2$. The direct action potential is practically unchanged, while the indirect action potential has disappeared and only an end-plate potential is observed. Horizontal calibration bar, 25.0 msec; vertical calibration bar, 50 mv for the voltage trace, and 8×10^{-7} ampere for the current trace. Tracings B_1 to B_6 are potentials from the same preparation as A. Tracing B_1 is a control nerve-induced action potential, and B_2, B_3, and B_4 were taken 1, 2, and 5 minutes, respectively, after the addition of 1.0 m*M* $MnCl_2$. After B_4 the $MnCl_2$ was washed from the preparation and B_5 and B_6 were taken 5 and 10 minutes afterward. They show a resumption of neuromuscular transmission. Horizontal calibration, 2 msec; vertical calibration, 25 mv.

The experiments were performed on a preparation of the frog sartorius neuromuscular junction in vitro; conventional methods were used for stimulation, intracellular recording, and averaging of signals (*5*). The bathing medium had a standard composition of 116.0 m*M* NaCl, 2.0 m*M* KCl, and 1.8 m*M* $CaCl_2$, unless otherwise mentioned. Manganese ions were added as an isotonic substitution for sodium. The resulting alteration in sodium concentration was too small to cause any significant change in quantal release of transmitter or postsynaptic sensitivity (*6*). Two different procedures were used to avoid contraction of the muscle and the subsequent dislodging of the microelectrodes; (i) procedure A was a detubulation of the muscle by osmotic shock (*7*), which interferes with the excitation-contraction coupling, and (ii) procedure B was a reduction in the concentration of calcium ions in the external solution (*8, 9*).

The inhibitory action of manganese ions on neuromuscular transmission is illustrated in Fig. 1 where procedure A was followed. Two microelectrodes were introduced into a muscle cell; one electrode for recording, and the second for intracellular stimulation. Muscle action potentials were elicited either directly by passing depolarizing currents through the second microelectrode, or indirectly by stimulating the nerve (Fig. $1A_1$). The addition of 1.0 m*M* Mn^{2+} abolished within a few minutes the muscle action potential evoked by nerve stimulation, but it left (Fig. $1A_2$) the "direct" action potential practically unchanged. Under these conditions, one can still observe a subthreshold end-plate potential. The action of manganese ions is reversible (Fig. 1B).

While the experiment illustrated in Fig. 1 shows that manganese ions are able to stop normal transmission across the neuromuscular junction, the experiment illustrated in Fig. 2 demonstrates that the main action of manganese ions is on the presynaptic membrane. The experimental method used was procedure B. When the calcium concentration was reduced to 0.4 m*M*, the end-plate potential amplitude fluctuated from one trial to another, according to the quantal hypothesis (*9*), and therefore mean amplitudes were obtained with an averaging computer (Fig. 2, a and c). The mean number of quanta of transmitter released by a nerve impulse (quantal content, *m*) was, under these conditions, 8.78. Addition of as little as 70 μM Mn^{2+} reduced the mean response to less than one-third of the control, the new quantal content being 2.38 (Fig. 2, b and d). The mean amplitude of the miniature end-plate potentials was practically unchanged. The results of this and 14 additional experiments show that manganese ions interfere with quantal release of transmitter from the motor nerve endings, following the nerve impulse.

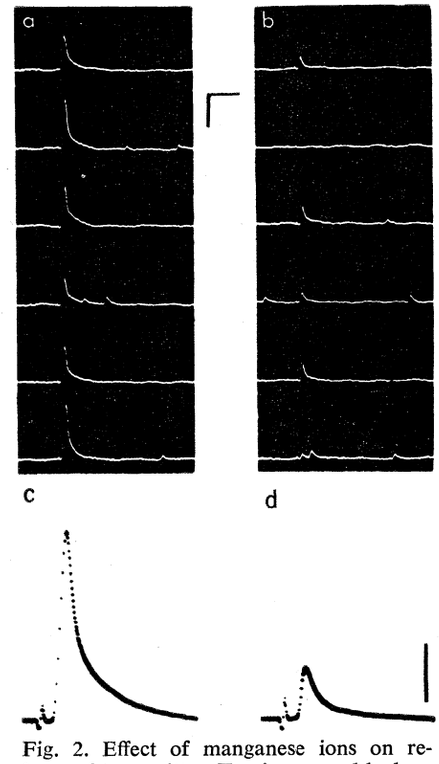

Fig. 2. Effect of manganese ions on release of transmitter. Tracings a and b show samples of individual responses, and c and d are automatically obtained averages of 200 responses. Stimulation frequency, 0.5 per second. Tracings a and c are control responses in 0.4 m*M* Ca^{2+} and 2.0 m*M* Mg^{2+}. Quantal content is 8.78. Tracings b and d were taken after the addition of 70 μM $MnCl_2$. The quantal content was reduced to 2.38. Vertical calibration, 1.0 mv for a and b; 0.3 mv for c and d. Horizontal calibration, 20 msec for a and b. Sampling frequency for c and d was 0.125 msec for each address.

A question arises as to whether some of the inhibitory action of manganese is due to its blocking effect on the action potential so that it cannot reach the terminal (*2*). If this is the case, then the number of trials with no response to stimulation should be larger than the number of these failures predicted by the Poisson theorem (*9, 10*). This point was checked in ten experiments and an agreement was found between the expected and the observed number of failures. For example, when a preparation was bathed in a Ringer solution containing 50 μM Mn^{2+}, 0.7 mM Ca^{2+}, and 1.0 mM Mg^{2+}, the mean amplitude of the end-plate potentials, on 195 trials, was 0.76 mv, and the mean amplitude of the miniature end-plate potentials was 0.35 mv, giving a quantal content of 2.17. The expected number of failures was 22.3 ($195e^{-2.17}$), while the observed number was 23.

The magnitude of the inhibiting action of Mn^{2+} ions depends on the concentration of Ca^{2+} in the extracellular medium; at higher Ca^{2+} concentrations, more manganese is needed to achieve the same fractional inhibition. This observation suggested that Mn^{2+} and Ca^{2+} may be competing for a common site on the presynaptic membrane. This point was further investigated by obtaining relations between Ca^{2+} concentrations and quantal content of transmitter at two different concentrations of Mn^{2+}. The modified Lineweaver-Burk plots [see (5)] had a common intercept, indicating that the inhibition is competitive in nature. Thus, the action of Mn^{2+} on transmitter liberation resembles that of magnesium (*11*). The main difference between these two ions is their potency, manganese being at least 20 times more potent on a molar basis. The qualitative similarity between Mn^{2+} and Mg^{2+} at the frog neuromuscular junction is not surprising in view of their resemblance in some physiochemical properties and their mutual replacement in a number of processes (*12, 13*).

The concentration of manganese in human extracellular fluid is estimated to be on the order of 1.0 μM (*13*), and, if the sensitivity of the various human synapses is similar to that of the frog neuromuscular junction, it is unlikely that under normal circumstances a significant fraction of transmitter release is inhibited by manganese. Recently it has been suggested that changes in manganese concentration in body fluids are associated with some neurological disorders (*15*). It would be of interest to see whether synapses exist, with high sensitivity to manganese ions, in the central nervous system.

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Deficient Activity of Hepatic Acid Lipase in Cholesterol Ester Storage Disease

Abstract. *Absence of lysosomal acid lipase activity in the liver is described in cholesterol ester storage disease and Wolman's disease. This enzyme deficiency may result in the excess hepatic cholesterol ester found in both conditions. However, clinical, genetic, and histopathologic differences suggest that the two conditions are separate diseases not completely explained by deficient enzyme activity.*

Cholesterol ester storage disease is a rare familial disorder of lipid metabolism characterized by asymptomatic hepatomegaly, normal liver function studies, and hypercholesteremia (*1–3*). The liver contains cholesterol esters at 100 to 500 times the normal concentration (*1, 4*). Neutral cholesterol esterase activity is normal (*3*). The ultrastructural appearance of hepatocytes indicates excessive lipid in membrane bound vacuoles (presumably abnormal lysosomes) (*2, 3*). We now describe deficiency of hepatic acid lipase (a lysosomal enzyme) activity in a previously reported patient (*3*). Liver biopsy for the determination of acid lipase activity was performed when the patient was 20 years of age. Total serum cholesterol was 275 mg per 100 ml of serum with 210 mg/100 ml as cholesterol ester.

Mahadevan and Tappel described a lysosomal acid lipase in rat liver and kidney with optimum activity at *p*H 4.2 (*5*). Using similar assay methods, Patrick and Lake reported lipolytic activity in human liver and spleen with optimum activity at *p*H 4.6 (*6*). More recent histochemical and ultrastructural studies provide evidence that human hepatic acid lipase is located in the lysosome (*7*).

Hepatic tissue was weighed, frozen on solid CO_2, and stored at −20°C or −75°C for less than 12 months before assay. Post-mortem specimens were obtained within 6 hours of death. Nitrogen was determined by the microkjeldahl method (*8*). The assay method for acid lipase was similar to that used by Patrick and Lake (*6*) (Table 1).

These findings indicate deficient activity of hepatic acid lipase in cholesterol ester storage disease. Patrick and Lake demonstrated a similar enzyme deficiency in Wolman's disease (*6*), an observation confirmed by our data. Activities of other lysosomal enzymes (that is, of β-galactosidase and of total hexo-